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- NEBuffer 1

Free Sample

Antarctic Phosphatase, a better enzyme than SAP at a better price. Add a Free Sample (#M0289G) to your order.

Exonuclease III (*E. coli*)

RR 37° Yes

Catalog #	Size	Concentration	Price	Qty	
M0206S	5,000 units	100,000 units/ml	$53.00	1	ADD TO CART
M0206L	25,000 units	100,000 units/ml	$212.00	1	ADD TO CART

Prices are in US dollars and valid only for US orders.

Download: MSDS PDF

- Isolated from a recombinant source
- 3'→5' exonuclease
- Produces unidirectional nested deletions
- Site-directed mutagenesis
- Supplied with 10X Reaction Buffer

Description:
Catalyzes the stepwise removal of mononucleotides from 3′-hydroxyl termini of duplex DNA (1). A limited number of nucleotides are removed during each binding event, resulting in coordinated progressive deletions within the population of DNA molecules (2).

The preferred substrates are blunt or recessed 3′-termini, although the enzyme also acts at nicks in duplex DNA to produce single-strand gaps. The enzyme is not active on single-stranded DNA, and thus 3′-protruding termini are resistant to cleavage. The degree of resistance depends on the length of the extension, with extensions 4 bases or longer being essentially resistant to cleavage. This property can be exploited to produce unidirectional deletions from a linear molecule with one resistant (3′-overhang) and one susceptible (blunt or 5′-overhang) terminus (3).

Exonuclease III activity depends partially on helical structure (4) and displays sequence dependence (C>A=T>G; ref. 5). Temperature, salt concentration and the ratio of enzyme to DNA greatly affect enzyme activity, requiring reaction conditions to be tailored to specific applications.
Exonuclease III has also been reported to have RNase H, 3′-phosphatase and AP-endonuclease activities (1).

Source:
Purified from E.coli K-12, BE257/pSGR3 strain (kindly supplied by B. Weiss)

Applications:
- Unidirectional nested deletions (3)
- Site-directed mutagenesis (6)
- Preparation of strand-specific probes (2)
- Preparation of single-stranded substrates for dideoxy sequencing (7)

Reagents Supplied:
NEBuffer 1 (10X)

Enzyme Properties

Nuclease Properties Comparison

Heat Inactivation:

70°C for 20 minutes

Specific Activity:
150,000 units/mg

Reaction & Storage Conditions

Reaction Conditions:
1X NEBuffer 1
Incubate at 37°C.

1X NEBuffer 1:
10 mM Bis-Tris-Propane-HCl
10 mM $MgCl_2$
1 mM dithiothreitol
pH 7.0 @ 25°C

Unit Definition:
One unit is defined as the amount of enzyme required to produce 1 nmol of acid-soluble total nucleotide in a total reaction volume of 50 µl in 30 minutes at 37°C in 1X NEBuffer 1 with 0.15 mM sonicated duplex [^{3}H]-DNA.

Concentration:
100,000 units/ml

Storage Conditions:
5 mM KPO_4
200 mM KCl
5 mM 2-Mercaptoethanol
0.05 mM EDTA
200 µg/ml BSA
50% glycerol
pH 6.5 @ 25°C

Storage Temperature:
-20°C

Notes

Usage notes:
1. Phosphorothioate linkages are not cleaved by Exonuclease III. Unidirectional deletions can also be created by protecting one terminus by incorporation of α-phosphorothioate-containing nucleotide (8).

FAQs

1. What is the activity of Exonuclease III in the NEBuffers?
2. Can DNA be blunted using Exonuclease III?
3. Why didn't the reaction using Exonuclease III work?
4. Why does all of the DNA get degraded in my Exonuclease III reaction?
5. How do T7 Exonuclease (NEB# M0263)and Lambda Exonuclease (NEB# M0262) differ from Exonuclease III?
6. Can Exonuclease III be heat inactivated?
7. What is the specific activity of Exonuclease III?

Quality Control

Quality Assurance Statement:
Purified free of contaminating endonucleases and exonucleases.

Endonuclease Activity:
Incubation of a 50 µl reaction containing 250 units of Exonuclease III (*E. coli*) with 1 µg of ΦX174 RF I DNA for 4 hours at 37°C resulted in < 50% conversion to RFII as determined by agarose gel

electrophoresis.

Quality control values for a specific lot can be found on the datacard which accompanies each product.

References

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Reagents Sold Separately

NEBuffer 1